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Using Bean Beetles (*Callosobruchus maculatus*) in Guided Inquiry Exercises in the Biology Laboratory

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Extended Abstract

Evidence supports the idea that guided inquiry exercises in biology laboratories are great alternatives to traditional exercises in which a student follows step-by-step instructions from a lab manual (for review, see National Research Council [US] Committee on Undergraduate Biology Education to Prepare Research Scientists for the 21st Century; Bio2010: Transforming Undergraduate Education for Future Research Biologists; National Academies Press [US], Washington DC, 2003). In a guided inquiry exercise, the instructor provides a context for a scientific study and perhaps an overarching question to be investigated. Within this context, the student then develops her own hypothesis, designs and performs her own experiment, analyzes the resulting data, and draws her own conclusions. Most instructors have students write the experiment up in a format that is similar to one used for a paper published in a scientific journal. Students (or student groups) may also be asked to present their experiment orally to the other students in the class. Guided inquiry exercises parallel more closely the work done by a practicing scientist than do traditional lab exercises. Furthermore, guided inquiry exercises challenge the student to think deeply about his project as he designs experiments and draws conclusions.

Due to institutional and instructor constraints, a guided inquiry exercise nearly always requires a "system" that can be manipulated by students. Reproducing bean beetles (*Callosobruchus maculatus*) constitute such a system. Details about the laboratory culture and life cycle of *Callosobruchus maculatus* are readily available, as are ideas for guided inquiry exercises using bean beetles (e.g., see www.beanbeetles.org). An overarching question to be investigated using this system might be, "What factors influence the reproductive success of bean beetles?" Typically, in a guided inquiry exercise, a student group will choose to test the influence of an experimental variable on a dependent variable. For example, students might hypothesize that living under conditions of high relative humidity (RH), compared to low RH, will result in an increase in (a) the mean number of eggs laid by newly mated virgin female beetles, (b) the mean number of days lived by newly emerged female beetles, (c) the mean time to emergence (TTE) of the offspring of newly mated female beetles (with TTE defined as the number of days elapsed between mating of the mother and emergence from the bean of her adult offspring), and/or (d) the emergence success (ES) of eggs laid by newly mated female beetles (with ES defined as the proportion of eggs laid resulting in successfully emerged adult offspring). As another example, students might hypothesize that being reared on mung beans, as opposed to on blackeye peas (BEP), will result in an increase in one or more of the above four dependent variables.

Materials that will be useful when using this system in guided inquiry exercises include (a) bean beetles (Carolina Biological Supply, Cat. No. 144180), (b) blackeye peas and mung beans (grocery stores; oriental grocery store may be necessary to find mung beans; some instructors prefer to buy organic beans to reduce potential pesticide residue, but we have had good luck rearing the beetles on nonorganic beans), (c) insect incubators maintained at high and low relative humidity (Carolina Biological Supply, Cat. No. 173150) (high RH incubator includes 150 mm plastic Petri dish kept full of water), (d) plastic Petri dishes, 60 mm x 15 mm, for holding beans and beetles, (e) hygrometers (available at electronics stores and home stores), (f) featherweight ("soft") forceps for safely handling beetles (Ward's Natural Science, Cat. No. 140520), and (g) dissecting microscope or magnifying glass for examining beans for eggs.

Procedures that students might follow when testing the hypotheses listed in the second paragraph of this abstract are as follows. From an existing bean beetle colony, place one-egg-bearing beans into small Petri dishes, one egg per dish. Check the dishes daily. When a female beetle emerges from a bean, record her day of emergence, assign her a number, and add a male beetle from an existing colony to her dish. Watch the beetles until they have mated, which usually only takes a few minutes. After mating, remove the male and cover the bottom of the dish containing the mated, numbered female with a layer of either BEP or mung beans. Repeat these steps with other females that emerge. Place half of the mated-female-on-BEP dishes in the high RH and half in the low RH incubator. Place half of the mated-female-on-mung-bean dishes in the high RH

and half in the low RH incubator. For each mated, numbered female, twice per week until she dies, count the number of eggs she lays. At each counting, remove any eggs (with their beans) into a separate small Petri dish labeled with the mother's identification number. Put this dish back into the same incubator that is holding the female whose eggs the dish contains. Examine the eggs laid by each mated, numbered female (that were put into their own Petri dish) every other day, recording whether or not an offspring has emerged from each of these eggs and, if so, how many days elapsed between mating of the mother and emergence of the offspring. Continue performing these steps until each mated, numbered female has died and until either all her eggs have resulted in emerged offspring or 55 days have passed since the female mated. Record the day on which each female dies and determine how many days she lived after emerging as an adult. For each of the four treatment conditions (BEP at high RH, BEP at low RH, mung beans at high RH, and mung beans at low RH), determine means for the following: days lived by the mated, numbered females, number of eggs laid by the mated, numbered females, mean number of days elapsed between mating of the numbered females and emergence of their offspring, and proportion of eggs laid by each mated, numbered female that resulted in successfully emerged offspring. Use a student's t test to compare high RH and low RH data regarding mean days lived, mean number of eggs laid, and mean time between mating of the mother and emergence from beans of her adult offspring (time to emergence, or TTE). Use a student's t test to compare BEP and mung bean data regarding mean days lived, mean number of eggs laid, and TTE. Use a binomial proportion test to compare high RH and low RH data regarding proportion of eggs laid by each female that resulted in successfully emerged offspring (emergence success, or ES). Use a binomial proportion test to compare ES on BEP with ES on mung beans. See Table 1 for example student data from beetles reared on BEP only.

Table 1. Example student data testing the influence of humidity on beetle reproduction. The members of any pair of numbers followed by the same superscripted letter are statistically different at $p < 0.05$.

	Mean days lived	Mean number of eggs laid	Mean time to emergence of offspring (days)	Emergence success (%)
BEP; high RH	14.7	69.8	34.3^{a}	79^{b}
BEP; low RH	13.3	69.1	36.2^{a}	73^{b}

Using the information presented above, an instructor can guide a student as she tests one of the hypotheses listed in the second paragraph of this abstract. Alternatively, with little additional knowledge or materials from the instructor, a student could test the influence of a different experimental variable, such as temperature or light-dark cycle, on one or more of the four dependent variables listed above.

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